DEPARTMENT OF DEFENSE
ARMED FORCES EPIDEMIOLOGICAL BOARD
5109 LEESBURG PIKE
FALLS CHURCH, VA 22041-3258

REPLY TO
ATTENTION OF

AFEB (15-1a)

17 September 1996

MEMORANDUM FOR THE SURGEON GENERAL, DEPARTMENT OF THE AIR FORCE
THE SURGEON GENERAL, DEPARTMENT OF THE ARMY
THE SURGEON GENERAL, DEPARTMENT OF THE NAVY

SUBJECT: AFEB Report on Health Effects of Low Level Exposure to Chemical Agents

1. At the request of the Assistant Secretary of Defense (Health Affairs), the Armed Forces Epidemiological Board (AFEB) recently reviewed the literature and provided recommendations for future work on the topic of long-term health effects associated with subclinical exposures to GB and Mustard. Their report is enclosed for your information. Please feel free to disseminate the report within your respective services.

2. If you have questions, please contact me at (703) 681-8012.

FOR THE ARMED FORCES EPIDEMIOLOGICAL BOARD:

Vicky L. Fogelman

VICKY L. FOGELMAN
Colonel, USAF, BSC
AFEB Executive Secretary

Encl

CF:
LtCol Parkinson

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Long-term Health Effects Associated with Sub-clinical Exposures to GB and Mustard

A Review Conducted by the Environment Committee
Armed Forces Epidemiological Board

Dennis M. Perrotta, PhD, CIC, Chair

July 18, 1996

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BACKGROUND

Recent evidence reviewed by the Department of Defense Persian Gulf Investigation Team suggested that one bunker in the Kamisiyah Ammunition Storage Depot in Southern Iraq may have held chemical weapons. U.S. soldiers from the 37th Engineer Battalion destroyed bunkers at this site in early March of 1991. No chemical agents were detected before, during, or after the demolition action. Troops were approximately three miles from the bunkers when they were destroyed. The possibility of chemical weapons being present was raised because shell fragments with polyethylene liners were discovered at this site by a United Nations investigative team. Such liners are used in chemical weapon delivery systems. No agent was reportedly detected by UN staff or U.S. troops at any time. No illnesses or signs or symptoms consistent with chemical agent exposure were reported by troops in the area at the time of the demolition action.

Despite the complete lack of confirmatory evidence, this information highlights the possible, though unsupported, concern regarding exposure of U.S. troops to chemical agents during DESERT STORM. In their search for adverse exposures and health outcomes in DESERT STORM participants, the Department is investigating all aspects of this, and other potential exposures and adverse health outcomes.

QUESTION

The Assistant Secretary of Defense (Health Affairs) has asked the Armed Forces Epidemiological Board (AFEB) to conduct a literature review and to critique and comment on the following question.

Are there observable long-term health effects associated with exposure to Sarin (GB) and mustard at concentrations below that needed to cause acute signs, symptoms, or injury?

The AFEB was directed to consider the question in general terms, not limited to any single incident.

METHODS

The question was assigned to the Environment Committee of the AFEB. The chair identified outside consultants knowledgeable in chemical weapons and/or toxicology and conducted telephone consultations and review. Literature searches were conducted and reviewed, and selected journal and book articles were obtained and critically reviewed. The vast majority of information was found in the open literature; three restricted reports were received and reviewed. Restricted information was safeguarded in accordance with policy and procedures.

FINDINGS

SARIN (GB)

GB is a nerve agent and is chemically known as isopropyl methyl phosphonofluoridate. It is a colorless and odorless liquid when pure; the vapor is also colorless. GB evaporates at approximately the same rate as water. Like other nerve agents (soman, tabun, VX), GB is a highly toxic organophosphate which irreversibly binds to acetylcholinesterase. As a result, acetylcholine accumulates at neuromuscular junctions and causes a loss of function at these junctions. This interferes with the fundamental mechanism required for the normal function of the central nervous system and the peripheral nervous system, that is, transmission of a nerve impulse. While the great majority of effects are due to the anticholinesterase actions of GB, not all effects are related to this characteristic.

Marrs, Maynard, and Sidell (1996) categorize the signs and symptoms of GB intoxication into three groups, muscarinic, nicotinic and central. The muscarinic signs and symptoms result from increased activity of the parasympathetic system and include miosis, dim vision, salivation, bradycardia, lacrimation, abdominal cramps; diarrhea and rhinorrhea. Nicotinic effects include pallor, tachycardia , hypertension, muscle fasciculation and weakness. Central nervous system effects include headache, anxiety, difficulty concentrating, restlessness, confusion, convulsions, and respiratory depression or paralysis, which can lead to death.

Signs and symptoms can be observed regardless of exposure route, but the intensity and sequence is influenced by the route of exposure. Skin exposure may cause localized sweating and fasciculations first. Vapor exposure where the eyes and respiratory tract may come into contact with GB first, may result in miosis, rhinorrhea, and tightness of chest first. Respiratory exposure appears to result in symptoms faster than skin exposure.

During vapor exposure studies and unintentional vapor exposures, the first signs and symptoms are usually miosis, rhinorrhea and/or chest tightness (Sidell, 1992). In fact, early studies often defined an individual as "exposed" when that person had at least one of these symptoms. Persons in the same area, without any health complaints, were not considered exposed or "hit." This is the first of a multitude of methodologic problems related to the question at hand. Only those who had clinical signs or symptoms would be studied and documented. Anyone else, even if they were in the same area, would not be considered exposed and would not be examined.

The amount of GB necessary to cause initial clinical signs and symptoms is debatable, but has been estimated to be approximately 2-3 mg-min/m^3. This is known as the Ct, or the concentration of agent vapor in air as milligrams per cubic meter times the time of

exposure in minutes (Sidell, 1992). McKee and Woolcott (1949) report that a single exposure to a Ct of 3.3 mg-min/m^3 (for 40 minutes) is the minimal dosage necessary to produce effects in men. However, they also state that chemical analysis of the agent indicated that the concentration actually given was approximately 75% of the intended dose, therefore the Ct would actually be approximately 2.5 mg-min/m^3. When the exposure time was reduced by 50% (20 minutes, Ct approximately 1.2 mg-min/m^3), a single dose did not produce symptoms, but the same report indicated that exposure to a Ct of 0.6mg-min/m^3 (1 minute) also caused detectable symptoms. It appears that a single exposure of man to a very small amount of GB will produce observable acute signs and/or symptoms. It is also important to note that an exposure with a Ct derived over a longer period of time (e.g. 240 minutes vs 20 minutes) will cause fewer or less severe signs and symptoms since there is some detoxification that occurs during the longer period of exposure.

As will be described below, there are essentially no controlled human studies in which men were exposed to doses calculated to avoid symptoms and where these men were followed over extended periods of time. Several studies utilizing doses that did cause acute symptoms, several unintentional high level exposure investigations, and animal studies can be used to make general suggestions regarding the long-term health risks associated with low-level exposure to GB.

Carcinogenicity, Mutagenicity, Teratogenicity

Organophosphates are not recognized as being carcinogens. No evidence was found to suggest that GB has carcinogenic potential. In a follow-up study of approximately 995 U.S. Army volunteers who participated in anticholinesterase experiments at the U.S. Army laboratories, Aberdeen Proving Ground, Edgewood, Maryland during 1955-1975, no consistent pattern of increased risk of cancer was found (NRC, 1985). The study was of relatively low statistical power, and was only able to identify large differences. The investigators concluded that, based on these findings, and the 10 lifetime studies of carcinogenicity of organophosphates sponsored by the National Cancer Institute, that anticholinesterase compounds did not induce malignancies among the Edgewood subjects.

Goldman, Klein, Kawakami and Rosenblatt (1987) concluded that GB is not mutagenic based on both *in vivo* and *in vitro* evaluations. Negative results were found in the Ames Salmonella bacterial gene mutation assay using 5 different strains exposed to a range of concentration of GB. Mouse lymphoma cell tests, Chinese hamster ovary cell tests, including sister chromatid exchange assays, and rat hepatocyte assays (for unscheduled DNA synthesis and damage) were all negative for mutagenic activity.

No evidence of teratogenicity of GB was found. Organophosphates are generally not considered to have significant reproductive effects; no studies to directly evaluate this

characteristic in GB were found. In their study of the toxicity of chronic exposure of dogs to GB, Jacobsen, Christensen, DeArmon, and Oberst (1959) had the male animals bred after 25 weeks of daily moderate doses of GB; the offspring were normal. In their one year, low-dose GB inhalation exposure study of a variety of animals, Weimer et al (1979) found no abnormalities in reproduction and fertility, fetal toxicity, or teratogenesis in Sprague-Dawley/Wistar rats. Testicular atrophy in was noted in the Fischer rat, but the authors speculated other causes, since later experiments (using a different route of exposure) did not replicate the finding. In their report, the authors also cite work conducted by J.R. Denk (EB-TR-74087 Effects of GB on Mammalian Germ Cells and Reproductive Performance, February 1975) which came to the same negative conclusions.

Neurotoxicity

Because GB is a nerve agent, the potential that exposure to GB would cause neurologic damage was reviewed. Many studies regarding neurotoxicity of organophosphate insecticides were reviewed, but in light of the varied differences between the insecticides and GB, they were not found to be particularly useful in this effort.

Human exposure to certain organophosphates has been related to a condition called "organophosphate-induced delayed neuropathy (OPIDN)." Weakness and ataxia develop in the lower limbs 8-14 days after acute poisoning with occasional progression to paralysis. This delayed neuropathy is associated with axonal degeneration and demyelination of peripheral nerves and certain parts of the central nervous system. The severity is related to the compound and the amount absorbed.

This condition has been substantially studied. Davies, Holland and Rumen (1960) found that GB at extraordinarily high doses (in excess of 60 x LD_{50})(LD_{50} is the dose that is lethal to half of the test population) caused delayed neuropathy in sensitive hens protected against the lethal effects by treatment with atropine and oxime P2S, a cholinesterase reactivator. Later, Johnson (1975) discovered that the ability of organophosphates to produce delayed neuropathy depends on their ability to organophosphorylate the enzyme neurotoxic esterase (NTE) in the nervous system. NTE can be used to more objectively study neuropathology related to chemical exposures.

Studies of the effects of GB appear conflicting, but this may be due to a large difference in dosage given to the hens. Gordon, et al (1983) confirmed that GB produced delayed neuropathy associated with high inhibition of NTE at levels as much as 60 x LD_{50}. Studies by Bucci, Parker and Gosnell (1992) indicated that doses around the LD_{50} did not result in any significant dose-related effects in the ataxia and neuropathy data. No histological evidence of neuropathy was observed in the treated hens, and there was

no significant inhibition of NTE activity, under the conditions of the study.

These two studies represent two very different points of the dose-response curve of GB as it relates to neuropathy. The exposures in both of these studies were very high; the lower dosage study found no neurotoxicity. These lower doses approximated the LD_{50}, and were much higher than the doses under consideration in this review. An earlier study (Crowell, Parker, Bucci and Dacre, 1989) also found no evidence that GB exposure at nonlethal doses caused any neuropathology.

Recently, Baker and Sedgewick (1996) examined subclinical changes at the neuromuscular junction of men exposed to moderate (enough to cause myosis and mild dyspnea) amounts of GB in test chambers. Using single fiber electromyography, the authors found small changes in the electromyography indicating the onset of neuromuscular block. In each case, the changes were reversed and were regarded as having no clinical importance.

Therefore, it appears that GB, at levels of concern in this review, does not cause delayed neuropathology.

Electroencephalographic Changes

Organophosphate compounds are known to have potent effects on the nervous system including electroencephalographic (EEG) changes. While most of the studies documented short term changes in EEG (Grob & Harvey 1953), Metcalf and Holmes (1969) suggest that organophosphate poisoning may lead to long-term EEG changes. Because of these findings, two major approaches to answering the question were begun; one in monkeys and another which examined unintentionally exposed workers in greater detail.

Burchfiel, Duffy and Sim (1976) exposed monkeys to GB at two dose schedules: a single high-level dose that produced convulsions in animals that required artificial respiration to prevent secondary brain anoxia, or a series of 10 smaller doses given at one week intervals (the report was vague about these low doses causing any symptoms). These monkeys were examined, EEGs were completed and then followed for a year, with EEGs completed at that time as well. All three of the "single high dose" animals, and two of three of the "series of low dose" animals exhibited statistically increased temporal lobe beta voltage one year after administration. None of the 10 control monkeys showed this difference ($p=0.039$, Fisher's exact).

Upon spectral analysis of the human EEGs, there was also increased beta activity in those exposed to GB (exposed group and maximally exposed group) as compared top the control group. Additionally, the investigators found an increase in REM (rapid eye movement) sleep in the exposed and maximally exposed group compared to the

controls. The authors were uncertain as to the true meaning, if any, as they might relate to changes in long-term brain function.

While these studies involve humans who were exposed to levels high enough to cause symptoms, and the animal studies had two dose regimens that had severe (high dose) and mild (low dose) signs and symptoms associated with them, they represent reasonable evidence that even small doses (exact level is unknown) may result in EEG changes. Exactly what, if anything, this means for the function and long-term health of the soldier is unclear.

Cardiomyopathy

One study of rats given various single doses of GB found various cardiac lesions upon light microscopy (Singer, Jaax, Graham, McLeod, 1987). Of those that survived the high dosages of GB, half had suffered convulsions, and most of those animals had microscopic evidence of brain damage, due perhaps to generalized hypoxia. Of all the animals with cardiac lesions, only one did not also have brain lesions. This study suggests that high dose GB can cause general hypoxia and neurogenic cardiomyopathy in rats. This evidence does not contribute to the issue of long-term health effects associated with sub-clinical dosage of GB. No evidence of cardiac problems was found related to GB.

General Health Measures

An examination of several follow-up measurements conducted on the men exposed to anticholinesterase chemicals (including GB) during experiments at Aberdeen Proving Grounds in Edgewood, Maryland did not find significant increases in hospital admissions, medical problems (self-reported), impairments, malignancies, or other adverse health outcomes (NRC, 1985). The expected "healthy soldier effect" was observed in some of the standardized morbidity (or mortality) ratios calculated in this effort. The authors admit to low-to-moderate statistical power to identify differences, therefore, only large differences would likely be uncovered.

Conclusions

Sarin, or GB is a highly toxic organophosphate nerve agent that can cause mild, reversible signs and symptoms at low doses, and death at doses that are not too much greater. There is no scientific information that directly answers the entire question: Are there observable long-term health effects associated with exposure to Sarin (GB) at concentrations below that needed to cause acute signs, symptoms, or injury? Extrapolation from a variety of sources, not designed to answer this particular question, was utilized. Some studies are clearly negative for a particular health effect at higher doses than that of concern to this question. These provide confidence that there is no

increased specific health risk at the low doses under question.

GB does not have carcinogenic or mutagenic properties. While the teratology literature is less clear, it appears that GB is not a teratogen. Therefore, no increase in birth defects or cancer would be expected from low-dose, short duration exposure to GB. Follow-up of a cohort of men exposed to GB found no significant increase in hospitalizations, reported health problems, mortality, or other measured end point.

There remains some question as to the validity of concerns regarding changes in EEG patterns long after GB exposure. Also, it is unclear what such changes really mean regarding the function of the soldier if those long-term EEG changes actually occur. It is prudent to suggest that further research into the long-term effects of low dose GB exposure (including doses that do not result in acute signs or symptoms) on the EEG of primates be undertaken.

Mustard (HD)

Mustard (also known as mustard gas, sulfur mustard, and in various forms, H, HD, HT) is *bis*(2-chloroethyl)sulfide, a chemical agent capable of producing severe chemical burns upon direct contact with tissue. Moist tissues such as the eyes and respiratory tract are especially vulnerable. Mustard was first used during World War I in great quantities. While it was not used militarily in World War II, there were exposures in December 1943 when an Allied ship carrying mustard munitions was attacked by German planes and exploded in the harbor of Bari, Italy. The explosion spread mustard over a wide area and caused hundreds of casualties. More recently, mustard has been used in the Iran-Iraq War (Borak & Sidell,1992).

Use of this vesicant chemical (referred to as HD in this report) is intended to decrease the opponents' ability to fight by producing burns and blisters on tissue, incapacitating the individual often for weeks (Watson & Griffin, 1992). The major routes of exposure include inhalation of vapors, and skin and eye contact with vapors or liquid droplets of HD.

Papirmeister (1991) describes the theoretical construct by which the mechanism of injury by HD occurs. Mustard is a potent alkylating agent. It rapidly alklyates the purine bases of DNA, which in turn activates endonucleases which remove alkylated bases. Removal of these bases creates places where the DNA breaks readily. The result is activation of the repair enzyme, poly(ADP-ribose) polymerase, which rapidly depletes cellular nicotinamide adenine dinucleotide (NAD^+). Depletion of NAD^+ inhibits glycolysis, leads to activation of tissue proteases, and results in cellular death. These effects do not appear immediately, but the latent period is related to concentration and duration of exposure. Mustard is a radiomimetic agent.

Clinically, high exposures cause eye irritation, blepharospasm, blurring of vision, pain and tissue damage. Contact of skin to vapor, mist, or droplets of HD results in death of the basal cells of the epidermis. Separation and increased permeability produces edema and leads to the characteristic blisters. These blisters begin as vesicles and then coalesce into bullae. There is also some inflammation at the site. Temperature and moisture of the skin impact absorption rates of HD by skin (Nagy, et al 1946). The eyes and respiratory system are affected at lower dosages than that necessary to elicit skin effects. The eye appears to require much more time to heal, and severe eye injury can occur before significant skin effects are seen.

Injury of the respiratory system is manifested with chest pain, cough, sore throat and hoarseness. Tachypnea and bronchospasm follow over the next 12 hours. Lethal exposures result in death from respiratory failure, secondary pneumonia, and occasionally, hemorraghic pulmonary edema (Papirmeister, et al 1991).

As will be described below, there are essentially no controlled human studies in which men were exposed to doses calculated to avoid symptoms and where these men were followed over extended periods of time. Several studies utilizing doses that did cause acute symptoms, several unintentional high level exposure investigations, and animal studies can be used to make general suggestions regarding the long-term health risks associated with low-level exposure to HD.

Carcinogenicity, Mutagenicity, Teratogenicity

The ability of HD to alkylate DNA strongly indicates that HD has carcinogenic properties. The International Agency for Research on Cancer concluded that available data were sufficient to support classification of mustard agent as a "group I" carcinogen (IARC, 1975). This category includes compounds for which a causal relationship between exposure and cancer can be substantiated. Toxicological studies in animals, and epidemiologic studies in man (battlefield exposures, unintentional exposures, and occupational exposures) all point to the ability of HD to cause respiratory cancer and skin cancer.

The Institute of Medicine of the National Academy of Sciences (IOM, 1993) has summarized a wide variety of animal studies which indicated that HD is a potent carcinogen. They include: pulmonary tumors in mice after intravenous injection, pulmonary tumors in mice after inhalation, skin malignancies after chamber exposures, and sarcomas after subcutaneous injections of mustard.

Similarly, that report provides overviews of the compelling epidemiologic evidence that HD is a human carcinogen. From the studies of workers at Japanese weapons factory workers of Ohkuno-jima and at a plant in Hiroshima, it is clear that occupational exposure (unmeasured, but likely to be at least moderate) to HD is associated with an

increase of respiratory cancer. Workers at another plant also had demonstrated excesses of laryngeal cancer. A second examination of the workers at the Okuno-jima plant demonstrated that the cancers were found in the "central, major airways, rather than in peripheral regions in the lungs." Additional studies of British workers, battlefield exposures (particularly difficult to interpret due to lack of information regarding age, smoking status, other chronic respiratory disease, etc), and follow-up of soldier volunteers at Aberdeen Proving Grounds experiments (NRC, 1985) all conclude similarly; there is an increased risk of cancer (mostly respiratory and skin) related to exposure to moderate to high doses of HD. Importantly, none of the studies were able to provide information to establish potential effects at low doses.

HD is considered a mutagenic agent. It has tested positive in a variety of test systems and mutations in Drosophila have included dominant lethal, phenotypic mutations, as well as recessive sex-linked, autosomal, and phenotypic lethal mutations (Fox & Scott, 1980). Sister chromatid exchanges have been noted in the lymphocytes of fisherman inadvertently exposed to mustard in discarded shells after World War II (Wulf et al., 1985). Rozimarek et al., (1973) found a significant difference in dominant lethal effects between control animals and those exposed to a high dose of HD vapor. Again, studies did not provide sufficient information to conclude if low-dose exposures would result in mutagenesis.

There do not appear to be clear lines of evidence in support of teratogenic properties of HD. Yamakido et al., (1985) studied electrophoretic patterns of plasma and erythrocyte proteins, general biochemical and health examinations of children of the former workers of the Ohkuno-jima poison gas plant. They found no examination values for the children which were significantly different from those of their parents. They concluded that no evidence for mustard induced mutations being detected in their group.

In animal studies, the evidence also fails to find HD teratogenic. In the same study noted above, Rozimarek et al., (1973) found that exposure of pregnant rats to the high dose of HD vapor was ineffective in increasing fetal toxicity or in producing gross teratogenic effects. Other studies found some effects, but generally at levels high enough to cause maternal toxicity; and such effects were regarded as resulting from the toxic impact on the mother, not the fetus. Hackett et al., (1987) administered HD to rats and rabbits via intragastric intubation at a variety of doses and found that HD was not teratogenic in rats and rabbits.

Respiratory Effects

Since inhalation of HD vapor is a signifcant pathway of exposure, the respiratory system is likely to be injured upon exposure. Papirmeister et al. (1991) demonstrated that inhalation of HD vapor primarily affected the laryngeal and tracheo-bronchial

mucosa. Morgenstern, Koss & Alexander (1946) describe a case-series of 10 patients who developed cough, hoarseness, sore throat, wheezing, dyspnea and other pulmonary complaints after working in a mustard plant. Most of them were followed for 2-4 years after their first exposure and their pulmonary complaints persisted to that point. Cough and weakness were hallmark chronic complaints.

Even early studies of U.S. servicemen who were exposed to HD during World War 1 (Berghoff, 1919 & Sandall, 1922) found long-term disability including shortness of breath, coughing, chest tightness and other bronchitic complaint and findings. Bronchial asthma, hyperreactivity to minor inhalation irritants, and increased risk of respiratory tract infections are also reported.

Easton et al. (1988) studied 511 workers of a British mustard manufacturing plant and found statistically significant excesses for asthma, bronchitis, influenza and pneumonia, and for non-malignant respiratory conditions in general. These excesses were found even among workers with less than three years of employment there.

While all of these reports thoroughly document long-term pulmonary effects after significant exposure to mustard, none of the studies provide useful information regarding the impact of very low level exposure to HD. All of the subjects sustained an initial acute injury with consistent signs and symptoms, and had experienced either a single high dose exposure, or multiple, repeated exposures to unknown, but likely smaller doses.

There is no direct evidence that answers the question of long-term respiratory effects in individuals who were exposed to very low levels of HD and suffered no acute respiratory tract injury. Such injuries may be minor and may be repaired. It could be argued that some clinically apparent injury would be necessary in order that chronic, long-term effects would be observed. The question of the long-term effect of a single, sub-clinical exposure to HD remains unanswered.

Ocular Effects

The eye is extremely sensitive to the effects of HD vapor or liquid. There is extensive data to demonstrate that severe burns of the eye are causally associated with long-term, ocular effects including keratitis and intractable, recurrent or prolonged conjunctivitis. Exposure of the eye to liquid HD can lead to perforation of the cornea and is much more dangerous than vapor exposure. As the severity of exposure increases, so does the likelihood of long-term injury.

Papirmeister et al., (1991) suggested that exposures less than 50-100mg-min/m^3 will cause simple conjunctivitis that will clear up within 2 weeks, but long-term follow-up analysis was not conducted. The Institute of Medicine (IOM, 1993) suggests that the

loss of ocular epithelium is a key factor in persistent defects of the cornea. This is also the case in alkali and acid burns of the eye.

No evidence was found to suggest that long-term ocular damage or disease would occur in the absence of an initial injury due to exposure to HD.

Skin Effects

The hallmark sign of HD injury is the blister on the skin of an individual exposed to liquid or vapor. After healing of that blister, residual cutaneous scars often occur. Novick et al.,(1977) demonstrated that skin cancers at the site of old scars occurs. It appears that cutaneous cancers following acute sulfur exposure usually occur at scar sites, where those occurring after chronic exposures can occur at any exposed site (Inada, et al., 1978). It appears that injury that results in erythema and edema without frank vesicle formation always heals without residual cutaneous effects.

The same authors identified pigmentary skin changes (either hyper-or hypo-pigmentation) on covered skin of nearly 25% of exposed workers at the Ohkuno-jima poison gas plant. Unfortunately, no information regarding exposure was provided, but it was assumed to be moderate-to-high.

Animal studies are of limited utility in studying the impact of HD on human skin, with the exception of studies of carcinogenesis detailed above. Human skin, and animal skin are different in important ways and there is no effective animal model for vesication. Humans appear to be more sensitive to mustard than any of the animal species tested.

There is ample evidence linking exposure of human skin to HD at levels high enough to cause acute injury, and the development of skin cancer, pigmentation disorders and skin ulcers. There is insufficient evidence to judge if exposures lower than that necessary to elicit an acute injury are associated with long-term skin problems and disease.

Other Effects

Immune System

Animals exposed to HD have shown changes in the cells of the immune system, with untoward effects, including immunosuppression, and altered host defense responses Coutelier et al., (1991). Extremely high human exposures that ultimately proved fatal have been studied and demonstrated dramatic reduction in leukocytes within one week (Stewart, 1918). More recently, during the Iran-Iraq war, infections were frequently found. The Institute of Medicine (IOM, 1993) cites an abstract that demonstrated depression of cell-mediated immunity up to three years after the exposure to HD. The

study group was made up of Iranians who suffered injury due to HD during the Iran-Iraq War.

It appears that high dose exposure of humans to HD can lead to alterations of immune function. No information was found regarding impact of very low dose exposure to HD on the immune system.

Psychological Aspects

A thorough literature review was not conducted on the potential long-term psychological effects of very low dose exposure to HD. The Institute of Medicine (IOM, 1993) conducted a good review on the relationship of exposure to psychological dysfunction as it pertains to experiences of men in chamber and field tests with HD. Their conclusion was that : "available evidence indicates a causal relationship between the experiences of the subjects in chamber and field tests of mustard agents and Lewisite and the development of adverse psychological effects. These effects may be individual, but diagnosable, and may include long-term mood and anxiety disorders, post-traumatic stress syndrome, or other traumatic disorder responses."

While the exposures appear different, there may be significant similarities between the situations within the report, and those in selected aspects of DESERT STORM. They are both outside the range of usual human experience. The report did not conclude that the chemical itself, and its effects on the human body, was particularly responsible for the relationship purported.

DISCUSSION

The long-term effects of limited exposures to sub-clinical doses of GB and HD are unclear, but the data included in this review suggest that health effects would not be detectable.

There are **NO** scientific data that directly apply to the question at hand, and precious little that indirectly address the fundamental question. All the human studies found and reviewed dealt with persons exposed (intentionally or unintentionally) and who reported signs, symptoms, or frank injury. Indeed, in most studies, the definition of "exposed" was the presence of clinical effects of any degree. This paucity of information is not unexpected. Most investigations were conducted when this country was investigating chemical weapons as a tool of offensive warfare, and was therefore interested in the large doses that might incapacitate opposing forces. One would expect little interest in low-dose exposures and sub-clinical effects under these circumstances.

The central concept of toxicological inquiry is the "dose." How much were individuals exposed to? And how long were they exposed, if they were? While the AFEB

understands that the request is hypothetical in nature, the inability to work with a dose level, greatly hinders any risk assessment methodology. During this review, the AFEB found several health effects for the two chemical agents that were clearly related to high level exposure. There was no useful methodology found that could be used to adequately extrapolate to the very low concentrations proposed in the question. The exceptions to that observation were those studies that were adequate to judge no effect at high doses. The AFEB concluded that there would be no effects at lower doses in these situations. The most critical limiting factor in this review was the fact that no reports of studies which examined health effects at sub-clinical doses were found.

The nature of an exposure in combat situations is not as simple as one might think. A large variety of factors can greatly impact the effective exposure that a soldier receives. Temperature, humidity, skin moisture, exposed surfaces, fit of personal protective equipment, pretreatment (in the case of nerve agents), wind strength and direction, whether the agent is in liquid or vapor form, activity level of the soldier (at rest or running), host susceptibility factors and other factors makes the estimation of a field does a very complex problem. A soldier that is "hit" with nerve agent (shows signs and symptoms of nerve agent exposure) may be standing close to another soldier who has no signs and symptoms at all.

A summary of the findings follows.

SARIN (GB): Multiple lines of evidence indicates that GB does not have carcinogenic, mutagenic or teratogenic properties. Therefore, no increases in birth defects or cancer would be expected from low dose, subclinical exposure to GB. Follow-up of a cohort of men exposed to GB found no increase in hospitalizations, reported health problems, mortality, or other measured end points.

Some information in humans and animals suggests that repeated low-dose exposures to GB could result in subtle, but measurable (upon spectral analysis) changes in the EEG of exposed animals and men. It is unclear whether the doses used resulted in "no", or "few" minor symptoms in animals, but the men did report minor effects consistent with GB exposure. The type of EEG changes were similar in the two groups; an increase in the relative amount of beta voltage was found up to one year post-exposure in the animals. The exposed group of men had significantly more beta voltage, relative to other voltage classes, than those who were not exposed to GB. The exposures were unintentional and occurred up to six years prior to the evaluation.

Neither the animal or human studies regarding EEG changes directly address the exact question. The similarity in findings between human and animal studies suggests that this may be a true effect. Whether the effect occurs in humans exposed to levels lower than that needed to cause acute signs or symptoms is unclear. Also uncertain is the clinical significance of this finding, if real, to the soldier. This area deserves continued

study, but the data are simply insufficient to recommend any additional action at this time.

MUSTARD (HD): This vesicating agent is well known to have carcinogenic potential, as it is a strong alkylating agent of DNA and RNA. This agent causes a variety of genetic lesions in many types of mammalian cells in a dose-response fashion. There is clear epidemiologic and toxicologic evidence that exposures to mustard high enough to cause acute symptoms either on the battlefield or in test chambers are associated with an increased risk of respiratory and skin cancers, and perhaps leukemia. This estimate is of unknown precision since exact exposure information is not available.

The risk of cancer related to mustard at dosages less than that necessary to cause any acute effects is much less clear. Carcinogenesis is clearly a dose-response phenomenon and very low exposures would have a very low increased risk associated with it. Additionally, the number of individuals exposed in any scenario of the Persian Gulf, would be relatively few, making it unlikely that a measurable increase in cancers could be detected. And finally, the length of exposure in these scenarios was extremely limited, as compared to the standard decades of daily exposures that are used in carcinogenic risk assessment.

Using standard cancer risk assessment methodology, an estimate for cancer risk was calculated. The US Environmental Protection Agency (1991) derives a unit risk of 8.5 $x10^{-2}$ per microgram/m^3 for mustard. Considering a single 5 minute exposure to HD at a concentration of 0.05 mg/m^3 (chosen to approximate a level 10% of a dose that might cause minimal signs and symptoms), the cancer risk was estimated as $5.8x10^{-7}$. This essentially means that for every 10 million persons exposed under these circumstances, 6 additional cancer cases would be expected to arise from this exposure. Since no DESERT STORM scenario included more that a few hundred to few thousand men at any one time, there would be no detectable additional cancer cases arising from this hypothetical scenario. It must be understood that changes in any of the assumptions of exposure will impact the final estimate and that this estimate was calculated with the understanding that no substantial evidence in support of exposure to HD during DESERT STORM was found.

While animal experiments indicate that mustard is a reproductive toxin at high doses, there is little human information available to evaluate this risk in humans, at high or low dose exposures. What little information was found suggested that HD was not teratogenic.

There is ample evidence to suggest that severe exposure of skin to HD is related to a variety of long-term skin ailments such as pigmentary disorders, skin ulcers, and cutaneous cancers. There is insufficient information to judge if exposures lower than that necessary to produce an acute effect will have a long-term adverse health result .

There is evidence that severe exposure of the eye to HD, with concomitant acute injury, is related to adverse long-term ocular conditions. No evidence of such an effect was found for exposures lower than that necessary to cause an acute injury. The data were very limited in this area and were insufficient to judge.

Exposure to high levels of HD causes significant damage to respiratory tissue and results in a variety of non-cancer respiratory conditions. There is no evidence that suggests short-term exposure to very low levels (less than necessary to cause any symptoms) of mustard is related to long-term health problems of the respiratory system. The data are very limited, and the theoretical possibility of long-term effects without acute injury can not be eliminated totally.

Immune function can be depressed or altered as a result of high dose exposure to HD. No convincing evidence was found that such alterations occur over the long-term as a result of exposure to concentrations less than that which causes acute signs or symptoms.

While a thorough literature search was not conducted on psychological aspects of chemical agent exposure, one reference had potential use for addressing the question. Psychological dysfunction was related to the circumstances surrounding exposures to mustard in test chambers and field trials. These circumstances may parallel those experienced by soldiers in selected areas of DESERT STORM. No conclusion is forwarded in this report.

RECOMMENDATIONS FOR FUTURE WORK

The greatest single problem in answering this question is the absence of information regarding health effects (or the lack thereof) of exposure to low dose of HD and GB. The purpose of research would be to determine what, if any, health effects occur upon exposures of varied lengths to sub-clinical levels of GB and HD. Of particular importance would be the effects of sub-clinical exposures to nerve agents on the central nervous system and the immune system, and the effect of HD on the eye and respiratory tract. One specific recommendation is to conduct sub-chronic, long-term inhalation studies with HD.

There are no "No Observable Effects Levels" (NOELS) established with any degree of confidence for any of the chemical agents. These NOELS would be useful for answering questions related to DESERT STORM, but also for establishing workplace and general population exposure limits for demilitarization efforts. Airborne dispersal of chemical agents appears to be the most likely route of exposure, so whole body inhalation studies might be first priority. Dermal contact should also be studied.

There continues to be concern regarding the health effects that may be associated with mixed exposures, and more information along these lines should be developed.

During this review, several papers mentioned the potential that susceptibility to chemical agents may vary among exposed individuals. There may be populations that are more susceptible to chemical agent effects than others, and this may bear further review.

The nerve agents are neurotoxicants, and while it is clear that they are not associated with delayed neuropathy, well-designed studies that examine the neurologic impact of low doses of nerve agents still need to be conducted. Such studies should include appropriate neurophysiological tests and follow current US Environmental Protection Agency Guidelines for Neurotoxicity Risk Assessment.

End of Report

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